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(54) Title: ISOLATED FrpB NUCLEIC ACID MOLECULE AND VACCINE			
(57) Abstract  The present invention provides an isolated nucleic acid molecule that encodes an amino acid sequence comprising a FrpB protein. The invention also provides vaccine compositions capable of protecting a mammal against infection by <i>N. gonorrhoeae</i> or <i>N. meningitidis</i> comprising the FrpB protein encoded by the isolated nucleic acid of the invention and a pharmaceutically acceptable carrier.			

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**ISOLATED FrpB NUCLEIC ACID MOLECULE AND VACCINE**

5 This invention was made in the course of work supported by Public Health Service  
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10 has certain rights in this invention.

**BACKGROUND OF THE INVENTION**

15 FrpB has been described as a 70 kD major iron-regulated, outer-membrane protein  
common to *N. gonorrhoeae* and *N. meningitidis* (16, 21). The iron uptake systems of *N.*  
*meningitidis* and *N. gonorrhoeae* are similar (3,17).

Previous studies showed that FrpB is surface exposed and immunogenic *in vivo* (1,16,  
20 41). Polyclonal and some monoclonal anti-FrpB antibodies recognize the denatured  
protein on Western blots of nearly all gonococcal and meningococcal isolates tested  
(16 and this invention). Other monoclonal antibodies directed against meningococcal  
FrpB are bactericidal and strain specific (41). Nevertheless, the size of FrpB appears  
to be well conserved.

25 FrpB is useful as a vaccine because of its surface exposure (1,16,41), partial antigenic  
conservation (8,16), and susceptibility to attack by bactericidal antibodies (41). The  
cloning and sequencing of the *frpB* gene of this invention has made possible the

production of a vaccine against infection in mammals by *N. gonorrhoeae* or *N. meningitidis*.

5

### SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid molecule that encodes an amino acid sequence comprising a FrpB protein.

- 10 The invention also provides a method of producing a vaccine composition that protects a mammal from infection by *N. gonorrhoeae* or *N. meningitidis* comprising combining the FrpB protein encoded by the isolated nucleic acid of the invention with a pharmaceutically acceptable carrier.
- 15 The invention further provides a vaccine composition capable of protecting a mammal against infection by *N. gonorrhoeae* or *N. meningitidis*, the vaccine composition comprising the FrpB protein encoded by the isolated nucleic acid of the invention and a pharmaceutically acceptable carrier.
- 20 In addition, the invention provides antibodies directed to an epitope of the FrpB protein encoded by the isolated nucleic acid sequence of the invention.

The invention also provides a method of detecting an antibody specific for *N. gonorrhoeae* or *N. meningitidis* in a sample comprising contacting the sample with a

- 25 FrpB protein encoded by the isolated nucleic acid sequence of the invention under conditions to form a complex between the polypeptide and the antibody; and detecting any complex so formed.

Furthermore, the invention provides a method of treating a mammal infected by *N.*

*gonorrhoeae* or *N. meningitidis* comprising administering to the mammal an antibody of the invention, wherein the antibody is directed to an epitope of an *N. gonorrhoeae* or *N. meningitidis* FrpB protein.

5

### **BRIEF DESCRIPTION OF THE FIGURES**

FIG. 1 Oligonucleotide MB.3 is shown 3' to 5' and corresponds to non-coding strand. The *frpB* sequence presented in this figure is deposited with GenBank under the accession number U13980.

10

FIG. 2 Restriction map of *frpB* clones. The position of the *frpB* ORF is indicated below the physical map by the stippled box. Only relevant cloning sites are shown C, *Cla* I; D, *Dra* I; E, *EcoR* I; M, *Mlu* I. Also shown is the position of oligonucleotide MB.3, which was deduced from the amino-terminal amino acid sequence of the mature protein.

15

FIG. 3 Nucleotide sequence of the gonococcal *frpB* gene from strain FA19. Single letter codes for deduced amino acid sequence are shown below the nucleotide sequence. Asterisk indicates termination codon. Solid bar below nucleotide sequence indicates putative Fur box. Putative -10 and -35 sequences are boxed. RBS indicates ribosome binding site. Solid triangle shows *Bgl* I site of  $\Omega$  insertion. Vertical arrow indicates signal peptidase I cleavage site. Inverted horizontal arrows indicate inverted repeat.

20

FIG. 4 Southern-blot analysis of FA19 and FA6807 DNA. Panel A was probed with pUNCH319-specific fragment. Panel B was probed with the  $\Omega$  fragment. Lanes 1 contain FA19 DNA digested with *Hinc*II and lanes 2 contain FA6807 DNA digested with *Hinc*II.  $\Omega$  fragment is 2kb. Molecular weight markers are shown in kilobases (kB).

25

FIG. 5 Western blot of FA19 and FA6807 membranes. Blot was probed with anti-FrpB monoclonal antibody, W.6. Lanes 1 and 2 are FA19; lanes 3 and 4 are FA6807. Lanes 1 and 3 contain total membranes prepared from iron-sufficient cultures; lanes 2 and 4 contain total membranes from iron-deficient cultures. Approximate locations of molecular mass standards are indicated at left in kilodaltons.

FIG 6 Growth of FA19 and FA6807 in CDM in the presence of variable concentrations of aerobactin. Graph A represents FA19; graph B represents FA6807. (filled-in  $\Delta$ ), 100uM citrate; (■), 2.5uM Tf; ( $\Delta$ ), 3uM aerobactin; (●), 1uM aerobactin; (□), 0.3uM aerobactin; and (○), no iron source.

FIG. 7  $^{55}\text{Fe}$  uptake from  $^{55}\text{Fe}$ -heme and  $^{55}\text{Fe}$ -Tf. Solid columns represent mean uptake from heme and open columns represent mean uptake from Tf. 100% uptake determined from average FA19 uptake experiment. Standard deviations are indicated by error bars. Genotypes are FA19 wild type, FA6807 (*frpB*), and FA6747 (*tpbA*).

FIG 8 Reconstruction of *frpB* in pACYC184. Relevant sites are B, *Bam*H I; C, *Cla* I; D, *Dra* I; M, *Mlu* I; and X, *Xba* I. Solid arrow represents chloramphenicol acetyl transferase (Cm), stripped arrow represents tetracycline resistance gene (Tc), solid bar represents pACYC184 origin of replication (Ori), stippled boxes represent *frpB* coding sequences, stippled arrow indicates entire *frpB* coding regions, open boxes represent DNA 5' and 3' of *frpB*. *frpB'* and *frpB''* represent partial *frpB* coding sequences.

FIG. 9 Growth of RK1065 (pACYC184) and RK1065 (pUNCH331) on heme plates. Plate 1 contains heme only. Plate 2 contains heme and d-aminolevulinic acid. A is RK1065 (pACYC184) and B is RK1065 (pUNCH331). Antibiotic discs are E., erythromycin; N, novobiocin; and R, rifampicin.

FIG. 10 Nucleotide sequence of the gonococcal *frpB* gene from strain FA1090. The three letter codes for deduced amino acid sequence are shown below the nucleotide sequence. Three asterisks indicate termination codon.

**DETAILED DESCRIPTION OF THE INVENTION**

The subject invention provides an isolated nucleic acid molecule that encodes an amino acid sequence comprising at least a portion of a FrpB protein. In one  
5 embodiment of this invention, the isolated nucleic acid molecule is DNA. In other embodiments of this invention, the isolated nucleic acid molecule is cDNA or RNA. In a preferred embodiment of this invention, the isolated nucleic acid molecule comprises a sequence that is the same as or substantially the same as at least a portion of the nucleotide sequence shown in Figure 3. In a more preferred embodiment, the isolated  
10 nucleic acid molecule comprises a sequence that is the same as the nucleotide sequence shown in Figure 3.

The invention also provides a FrpB protein comprising the amino acid sequence encoded by the isolated nucleic acid molecules described above. Preferably, the  
15 amino acid sequence encodes an antigenic, and more preferably, an immunogenic FrpB. As used herein, antigenic means that the FrpB induces specific antibodies in a mammal, and immunogenic means that the FrpB induces an immune response in a mammal.

20 As used herein, the term "FrpB" means Fe-regulated protein B and encompasses any polypeptide having an amino acid sequence identical, or substantially identical, to the amino acid sequence of a naturally-occurring FrpB, as well as antigenic fragments thereof. The FrpB nucleic acid and amino acid sequences in the various strains of *N. gonorrhoeae* and *N. meningitidis* are homologous, but exhibit slight differences in their  
25 sequences, for example, the nucleic acid and amino acid differences between the homologous strains FA19 and FA1090 shown in Figure 3 and Figure 10, respectively.

In addition, FrpB encompasses equivalent antigenic polypeptides whose amino acid sequence varies from a naturally-occurring FrpB by one or more amino acid, either



internally such as a point mutation, or by addition or deletion at the COOH<sup>-</sup> terminus or NH<sub>2</sub> terminus or both. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by one or more substitutions, additions and/or deletions, is considered to be an equivalent sequence. Preferably, less than 25%.  
5 more preferably less than 10%, and most preferably less than 5% of the number of amino acid residues in a sequence are substituted for, added to, or deleted from the proteins of the invention.

For example, it is known to substitute amino acids in a sequence with equivalent amino  
10 acids. Groups of amino acids generally considered to be equivalent are:

- (a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
- (b) Asn(N) Asp(D) Glu(E) Gln(Q);
- (c) His(H) Arg(R) Lys(K);
- 15 (d) Met(M) Leu(L) Ile(I) Val(V); and
- (e) Phe(F) Tyr(Y) Trp(W).

Such FrpB equivalents include analogs that induce an immune response in a mammal  
20 comparable to that of natural FrpB. In addition, such equivalents are immunologically cross-reactive with their corresponding FrpB protein.

A FrpB protein fragment preferably contains sufficient amino acid residues to define an epitope of the antigen. The fragment may, for example, be a minigene encoding only the  
25 epitope. Methods for isolating and identifying immunogenic fragments from known immunogenic proteins are described by Salfeld et al. (72) and by Isola et al. (73).

If the fragment defines a suitable epitope, but is too short to be immunogenic, it may be conjugated to a carrier molecule. Some suitable carrier molecules include keyhole limpet

hemocyanin, Ig sequences, TrpE, and human or bovine serum albumen. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

- 5 In a preferred embodiment, FrpB of FA19 is or is an equivalent of the approximately 73 kD outer membrane FrpB protein that is part of the iron regulon of *Neisseria gonorrhoeae* or of *Neisseria meningitidis*. Determinations whether two amino acid sequences are substantially homologous may be based on FASTA searches in accordance with Pearson and Lipman (74).

10

The FrpB of the present invention may be prepared by methods known in the art. Such methods include, for example, (a) isolating FrpB directly from *Neisseria gonorrhoeae* or *Neisseria meningitidis*; and (b) using the nucleic acid molecule of the invention encoding FrpB to produce recombinant FrpB.

15

(a) Direct Isolation of FrpB:

- The FrpB may be isolated directly from *Neisseria gonorrhoeae* or *Neisseria meningitidis* by methods known in the art. First, gonococcal or meningococcal outer membranes are isolated and prepared by known methods. The methods described by West and Sparling (75) and by Schryvers and Morris (76) are suitable.

20

- The isolated membrane FrpB proteins or fragments may be solubilized by known methods, such as the addition of detergents. Commonly used detergents include Octyl-B-Glucoside, Chaps, Zwittergent 3.14 or Triton-X. The use of detergents to enhance solubility of membrane proteins is described by Jones et al. (77), Helenius et al. (78), and Hjelmeland and Chrambach (79).

25

The FrpB proteins or fragments are isolated from the solubilized membrane fraction by standard methods. Some suitable methods include precipitation and liquid

chromatographic protocols such as ion exchange, hydrophobic interaction and gel filtration. See, for example, Methods Enzymol. (80) and Scopes (81).

5 Purified material may also be obtained by separating the protein or fragment on preparative SDS-PAGE gels, slicing out the band of interest and electroeluting the protein from the polyacrylamide matrix by methods known in the art. The detergent SDS is removed from the protein by known methods, such as by dialysis or the use of a suitable column, such as the Extracti-Gel column from Pierce.

10 (b) Using Nucleic Acid Molecule of the Invention to Produce FrpB:

Alternatively, recombinant methods known in the art may be used for preparing FrpB. For example, FrpB may be produced from the isolated or synthesized nucleic acid molecule of the invention that encodes at least a portion of FrpB; cloning the DNA in a suitable host; expressing the DNA in the host; and harvesting FrpB. (See Sambrook et  
15 al. (82)).

Using standard methods of nucleic acid isolation, DNA can be obtained from strains that have been deposited with the American Type Culture Collection, Rockville, Maryland. FA1090 (ATCC Accession No. ) was deposited on April 8, 1996, in  
20 accordance with the Budapest Treaty. Strain FA19 (ATCC Accession No. 55073) was deposited earlier on July 12, 1996, also in accordance with the Budapest Treaty.

The DNA may also be synthesized chemically from the four nucleotides in whole or in part by methods known in the art. Such methods include those described by Caruthers in  
25 Science 230, 281-285 (1985).

If necessary a full length DNA may also be produced by preparing overlapping double-stranded oligonucleotides, filling in the gaps, and ligating the ends together. The DNA may be cloned in a suitable host cell and expressed. The DNA and protein may be recovered

from the host cell. See, generally, Sambrook et al, "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987).

- 5 The invention provides a vector which comprises the nucleic acid molecule described above which encodes an amino acid sequence comprising at least a portion of FrpB. Suitable vectors comprise, but are not limited to, a plasmid or a virus. This vector may be transfected into a suitable host cell to form a host vector system for the production of FrpB or of a polypeptide having the biological activity of at least a portion of a FrpB  
10 antigenic polypeptide.

Cloning vectors may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, and RP4. Prokaryotic vectors also include  
15 derivatives of phage DNA such as M13, f1, and other filamentous single-stranded DNA phages.

- Vectors for expressing proteins in bacteria, especially E.coli, are also known. Such vectors include pK233 (or any of the tac family of plasmids), T7, and lambda P<sub>L</sub>.  
20 Examples of vectors that express fusion proteins include the PATH vectors described by Dieckmann and Tzagoloff (83). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); maltose binding protein (pMAL); and glutathione S-transferase (pGST) - see Gene (84) and Peptide  
25 Research (85).

Vectors useful in yeast are available. A suitable example is the 2μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-

known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and vectors derived from combination of plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg (86); S. Subramani et al (87); R.J. Kaufmann and P.A. Sharp (88); S.I. Scahill et al (89); G. Urlaub and L.A. Chasin (90).

The expression vectors preferably contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of f1 coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters of SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Suitable expression hosts include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHI, and E. coli MRCI, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces. Suitable eukaryotic cells include yeasts and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

### VACCINES

FrpB encoded by a nucleic acid molecule of this invention has particular utility as a vaccine that protects a mammal from infection by *N. gonorrhoeae* or *N. meningitidis*, since the FrpB unexpectedly induces an effective immune response when presented to the immune system that protects from or prevents infection by *N. gonorrhoeae* or *N.*

5 *meningitidis*. To protect from infection by *N. gonorrhoeae*, the FrpB is preferably substantially the same, as defined above, as at least a portion of the FrpB of *N. gonorrhoeae*. To protect from infection by *N. meningitidis*, the FrpB is preferably substantially the same, as defined above, as at least a portion of the FrpB of *N. meningitidis*. The immune response may also produce a therapeutic effect in an already  
10 infected mammal. The mammal is preferably a human.

The invention provides a vaccine composition which comprises the FrpB protein encoded by a nucleic acid of the invention and a pharmaceutically acceptable carrier, such as saline, sterile water, phosphate buffered saline solution, liposomes and emulsions.

15 Other buffering and dispersing agents and inert non-toxic substances suitable for delivery to a mammal may be incorporated in the vaccine composition and are well known to those skilled in the art. The compositions may be sterilized by conventional sterilization techniques.

20 Adjuvants, which facilitate stimulation of the host's immune response, may be used in the vaccine compositions. Such adjuvants may include, for example, muramyl peptides, lymphokines, such as interferon, interleukin-1 and interleukin-6, or bacterial adjuvants. The adjuvant may comprise suitable particles onto which the mutant or wild-type FrpB protein is adsorbed, such as aluminum oxide particles. These vaccine compositions containing  
25 adjuvants may be prepared as is known in the art.

The concentration of FrpB in the composition may vary depending on, for example, fluid volume or antigenicity, and in accordance with the particular mode of administration chosen.

The invention further provides a method of protecting a mammal against infection by *N. gonorrhoeae* or *N. meningitidis* comprising administering to the mammal the vaccine composition of the invention. The vaccine may be administered to a mammal by methods known in the art. Such methods include, for example, oral, intravenous, intraperitoneal, subcutaneous, intramuscular, topical, or intradermal administration.

This invention also provides a method of producing the above vaccine composition by combining FrpB with a pharmaceutically acceptable carrier, and preferably, also with an adjuvant, as defined above.

10

### FrpB ANTIBODIES

The invention provides antibodies raised against FrpB epitopes encoded by at least a portion of the isolated nucleic acid sequence of the invention. The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein (91) and the recombinant DNA method described by Huse et al. (92).

Mammals infected with *N. gonorrhoeae* or *N. meningitidis* may be treated by administering an antibody of the invention. Preferably, an antibody raised against a polypeptide comprising an amino acid sequence present in *N. gonorrhoeae* or *N. meningitidis* is preferred.

For therapeutic purposes, the antibodies are preferably neutralizing antibodies that

significantly inhibit the growth of or kill the bacterial cells *in vitro* or *in vivo*. Growth of the bacteria is significantly inhibited *in vivo* if the inhibition or neutralization is sufficient to prevent or reduce the symptoms of the disease of a mammal infected with the disease.

5

Neutralizing antibodies may also be used to produce anti-idiotypic antibodies useful as vaccines for immunizing mammals infected with *N. gonorrhoeae* or *N. meningitidis*. Anti-idiotypic antibodies are prepared in accordance with methods known in the art.

10

#### **DETECTING FrpB USING PROBES**

The invention also provides a method of detecting FrpB in a sample using a probe specific for a FrpB polypeptide. The probe may be an antibody described above.

Methods are known for detecting polypeptides with antibodies. For example, a

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polypeptide may be immobilized on a solid support. Immobilization of the polypeptide may occur through an immobilized first antibody specific for the polypeptide. The immobilized first antibody is incubated with a sample suspected of containing the polypeptide. If present, the polypeptide binds to the first antibody.

20

A second antibody, also specific for the polypeptide, binds to the immobilized polypeptide. The second antibody may be labeled by methods known in the art. Non-immobilized materials are washed away, and the presence of immobilized label



indicates the presence of the polypeptide. This and other immunoassays are described by David, et al., in U.S. Patent 4,376,110 assigned to Hybritech, Inc., La Jolla, California.

- 5 The probe may also be a nucleic acid molecule that recognizes a FrpB nucleic acid molecule of the invention. Methods for determining whether a nucleic acid molecule probe recognizes a specific nucleic acid molecule in a sample are known in the art. Generally, a labeled probe that is complementary to a nucleic acid sequence suspected of being in a sample is prepared. The presence of probe hybridized to the target
- 10 nucleic acid molecule indicates the presence of the nucleic acid molecule. Suitable methods are described by Schneider et al in U.S. Patent 4,882,269, which is assigned to Princeton University, and by Segev in PCT Application WO 90/01069, which is assigned to ImClone Systems Incorporated.
- 15 The probes described above are labeled in accordance with methods known in the art. Methods for labeling antibodies have been described, for example, by Hunter and Greenwood (93) and by David et al. (94). Additional methods for labeling antibodies have been described in U.S. patents 3,940,475 and 3,645,090. Methods for labeling oligonucleotide probes have been described, for example, by Leary et al (95); Renz
- 20 and Kurz (96); Richardson and Gumport (97); Smith et al. (98); and Meinkoth and Wahl (99).

The label may be radioactive. Some examples of useful radioactive labels include  $^{32}\text{P}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^3\text{H}$ . Use of radioactive labels have been described in U.K. 2,034,323, U.S. 4,358,535, and U.S. 4,302,204.

5

Some examples of non-radioactive labels include enzymes, chromophors, atoms and molecules detectable by electron microscopy, and metal ions detectable by their magnetic properties.

- 10 Some useful enzymatic labels include enzymes that cause a detectable change in a substrate. Some useful enzymes and their substrates include, for example, horseradish peroxidase (pyrogallol and o-phenylenediamine), beta-galactosidase (fluorescein beta-D-galactopyranoside), and alkaline phosphatase (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium). The use of enzymatic labels have been
- 15 described in U.K. 2,019,404, EP 63,879, and by Rotman (100).

- Useful chromophores include, for example, fluorescent, chemiluminescent, and bioluminescent molecules, as well as dyes. Some specific chromophores useful in the present invention include, for example, fluorescein, rhodamine, Texas red,
- 20 phycoerythrin, umbelliferone, and luminol.

The labels may be conjugated to the antibody or nucleotide probe by methods that are well known in the art. The labels may be directly attached through a functional group on the probe. The probe either contains or can be caused to contain such a functional group. Some examples of suitable functional groups include, for example, amino,  
5 carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate.

The label may also be conjugated to the probe by means of a ligand attached to the probe by a method described above and a receptor for that ligand attached to the label. Any of the known ligand-receptor combinations is suitable. The biotin-avidin  
10 combination is preferred.

The polypeptide of the invention may be used to detect the presence of antibodies specific for *N. gonorrhoeae* or *N. meningitidis* in a sample. The method comprises preparing a polypeptide containing a segment having an amino acid sequence that is  
15 substantially the same as a FrpB from either *N. gonorrhoeae* to detect antibodies to *N. gonorrhoeae* or *N. meningitidis* to detect antibodies to *N. meningitidis*. The polypeptide may be prepared as described above.

The sample may, for example, be from a patient suspected of being infected with *N. gonorrhoeae* or *N. meningitidis*. Suitable assays are known in the art, such as the  
20 standard ELISA protocol described by R.H. Kenneth (101).

Briefly, plates are coated with antigenic polypeptide at a concentration sufficient to bind detectable amounts of the antibody. After incubating the plates with the polypeptide, the plates are blocked with a suitable blocking agent, such as, for example, 10% normal goat serum. The sample, such as patient sera, is added and titrated to determine the endpoint. Positive and negative controls are added simultaneously to quantitate the amount of relevant antibody present in the unknown samples. Following incubation, the samples are probed with goat anti-human Ig conjugated to a suitable enzyme. The presence of anti-polypeptide antibodies in the sample is indicated by the presence of the enzyme.

The following Examples section is set forth to aid in an understanding of the invention. This section is not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

### EXAMPLES

**Strains and growth conditions.** Bacterial strains used in this experiment are described in Table 1. *Neisseria* strains were routinely cultured on GCB media (Difco Laboratories) containing Kellogg's supplements I and II (29) and grown overnight at 35°C in an atmosphere of 5%CO<sub>2</sub>. Antibiotic selection employed chloramphenicol at 1µg/ml for mTn3(Cm)(51) mutagenized strains and streptomycin at 100µg/ml for Ω (44) mutagenized strains.

For western blot analysis of total membrane proteins of iron-stressed gonococci, cells

were grown in CDM as previously described (13). Cultures were made iron replete as indicated by the addition of 100uM ferric citrate.

*E.coli* strains were routinely cultured on Luria-Bertani (LB) media (47). Antibiotic selection was 100µg/ml ampicillin, 100µg/ml streptomycin, 40µg/ml kanamycin, and/or 30µg/ml chloramphenicol.  $\delta$ -aminolevulinic acid was used at 30µg/ml and heme at 50µg/ml. *E.coli* cultures were iron stressed by the addition of 200µM 2,2-diylridyl (Sigma Chemical Co., St. Louis, MO). Deferoxamine mesylate (desferal) was obtained from Ciba-Geigy (Basel, Switzerland).

**SDS-PAGE and Western Blotting.** SDS-PAGE was performed in 7.5% polyacrylamide resolving gel and 4.5% polyacrylamide stacking gel. Electrophoresis was carried out at either 40 mA for one gel, or 80 mA for two gels in the discontinuous buffer system of Laemmli (32). Transfer and development were as described previously (23,61).

**Preparation of polyclonal antisera and monoclonal antibodies.** Preparation of polyclonal antisera was described previously (8). Anti-FrpB monoclonal antibodies were generated by methods described previously (60).

**DNA isolation, digestion, and Southern blot analysis.** Chromosomal DNA was purified by CsCl1-gradient centrifugation according to the methods of Stern et al. (54). Plasmids were purified by either CsCl1 centrifugation or according to the instructions provided in the Magic Miniprep<sup>TM</sup> DNA Purification Kit (Promega; Madison WI). Southern blotting and DNA hybridizations were performed as previously described (13). Restriction enzymes, Klenow fragment of DNA polymerase I, and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA) or Bethesda Research Laboratories (Gaithersburg, MD) and were used according to the manufacturer's

specifications.  $\lambda$ -ZapII and pBluescript II SK+ were obtained from Stratagene (La Jolla, CA).

- DNA sequencing and sequence analysis.** CsCl-purified pUNCH319 and pUNCH325 were used as templates for double-stranded DNA sequencing (31) using United States Biochemical Sequenase and the dideoxy chain termination procedure of Sanger et al. (48). Both dG- and dI- labeling reactions were carried out for all primers. Both strands of pUNCH319 were sequenced using vector-specific or insert-specific primers. Exonuclease III/Exo VII nested deletions (40) were generated from the *Mlu* end of pUNCH325 and vector-specific primers were used to sequence individual deletion clones. Internal primers were used to sequence gaps between clones as well as the opposite strand. DNA sequences were analyzed with the Genetics Computer Group software package (15) (University of Wisconsin).
- Mutagenesis and gonococcal transformation.** pHP45 $\Omega$  (44) was used to insert ionally inactivate *frpB*. pUNCH321 was digested with *Bgl*I and ends were repaired with Klenow. pHP45 $\Omega$  was digested with *Sma*I and the 2.0kb  $\Omega$  fragment was isolated from an agarose gel according to the instructions provided in the GeneClean II Kit (Bio101 Inc. La Jolla, CA). Transformation of plasmid DNA into FA19 was as previously described (7).

- Preparation of FrpB for amino-terminal sequence analysis.** N-lauroylsarcosine (Sigma) insoluble membrane fractions were prepared from iron-stressed gonococcal strain UU1008 and protein concentration was determined by a bicinchoninic acid assay (BCA) (Pierce, Rockford, IL). Two hundred micrograms of protein was loaded into a preparative well of a 7.5% SDS-polyacrylamide gel, poured 24 hours previously to permit TEMED (N,N,N',N'-tetramethylethylenediamine) and APS (ammonium persulfate) to evaporate. Electrophoresis was carried out at 40 mA constant current

using the discontinuous buffer system of Laemmli (32). The gel was soaked for 15 minutes in transfer buffer (13) before transferring. PVDF (polyvinylidene difluoride) membrane was placed in 100% methanol for two seconds, transferred to distilled deionized water (ddH<sub>2</sub>O) for five minutes, and soaked in transfer buffer for 10 minutes prior to transfer. Transfer was for three and a half hours at 90mA in a submerged trans-blot apparatus (BioRad, Richmond, CA). Subsequent to transfer, the PVDF membrane was stained for five minutes in 0.1% Coomassie Brilliant Blue, 20% methanol, and 10% acetic acid to visualize proteins and destained for 10 minutes in ddH<sub>2</sub>O with one change. Filter was frozen at -20°C overnight. FrpB was identified by molecular weight and the amino-terminal amino acid sequence of the protein on the filter was determined by the Protein Microsequencing Facility at UCLA.

**<sup>55</sup>Fe uptake assays.** Data were compiled from three individual experiments performed in triplicate on separate days. Gonococci were iron stressed as previously reported (2) prior to experimentation. SDS-PAGE and Western blotting of whole-cell lysates were routinely performed to determine that cultures were consistently and equivalently iron stressed, as evidenced by reactivity with anti-FrpB monoclonal antibody and/or anti-Tbp1 antisera. Iron-uptake assays were performed as previously reported (9) with the following modifications. Filters were blocked just prior to experimentation with 30μl, 10mg/ml BSA in 1XCDM. Assays were performed in 200μl volumes in 96 well filtration plates (MAHV Millipore, Bedford, MA) at 35°C in a 5% CO<sub>2</sub> atmosphere. Potassium cyanide was dissolved in 1XCDM. The vacuum manifold was from Millipore Multiscreen Assay System. Heme was used at 0.5μM, transferrin at 6.25μM, and citrate at 100μM. Membranes were air dried overnight, and the Millipore punch kit was used to separate and collect individual filters prior to counting. Data were expressed as counts per minute per μg of protein.

**Preparation of aerobactin and enterobactin.** Purified aerobactin and enterobactin

were the generous gift of P.E. Klebba. Aerobactin was ferrated as follows. Ferric sulfate was dissolved to 4mM in 50ml ddH<sub>2</sub>O containing 1.5μl HCl. 400μl 4mM aerobactin was added to 400μl 4mM ferric sulfate and 80μl 0.5M Na<sub>2</sub>HPO<sub>4</sub>. The ferri-aerobactin was run over a CM-cellulose (Sigma, St. Louis, MO) column equilibrated in 0.05M Na<sub>2</sub>HPO<sub>4</sub>. The final concentration of aerobactin was determined by reading the absorbance at 400nm (24).

**Iron sources.** Human transferrin, human lactoferrin, bovine heme, human hemoglobin, and human haptoglobin were obtained from Sigma Chemical Co. (St. Louis, MO). <sup>55</sup>Fe hemin was purchased from the custom synthesizing facility at NEN Products Dupont (Wilmington, DE) lot number FE55.1193RS. Transferrin, lactoferrin, and citrate were ferrated with <sup>55</sup>FeCl<sub>3</sub> as previously described (36).

**RNase assay.** The RNase assay was performed as previously described (71), except 0.1N HCl was used instead of 0.5N HCl.

**Hemin affinity purification.** Hemin agarose was purchased from Sigma Chemical Co. (St. Louis, MO). The method of affinity purification was described by Lee (33).

**Bactericidal assays.** Bactericidal assays were performed as described previously (18).

**Cloning the gonococcal *frpB* gene.** Sarcosyl insoluble membrane fractions from gonococcal strain UU1008 were used to obtain FrpB N-terminal amino acid sequence (see above). A degenerate oligonucleotide containing inosine (designated MB.3, shown in Fig. 1) was deduced from this sequence and used to probe a Southern blot of FA19 chromosomal DNA. Each restriction digest contained a single hybridizing band. A 5.8kb *Dra* I fragment was chosen for further analysis.



A  $\lambda$ -ZapII library containing *EcoRI*-linkered FA19 chromosomal *Dra* I fragments (2) was screened with oligo MB.3. Approximately one positive plaque was identified for every 10,000 plaques screened. Attempts to excise the phagemid containing the intact insert consistently resulted in deletion products smaller than pBluescript II SK<sup>+</sup> alone. Since such a large chromosomal fragment potentially contained both the *frpB* promoter and entire *frpB* coding sequence and that the expression of FrpB might be toxic in *E.coli*, smaller fragments were subcloned into pBluescript II SK<sup>+</sup>.

DNA prepared from one of the positively hybridized plaques,  $\lambda$ frpB-4(Fig. 2), was digested with *EcoRI* to release the insert DNA. The expected 5.8kb fragment was isolated from an agarose gel and further digested with *Cla* I to generate a 540bp, MB.3-hybridizing fragment and an approximately 5.3kb fragment which did not hybridize to MB.3. The smaller fragment ligated into pBluescript II SK<sup>+</sup> was stable in *E.coli* DH5 $\alpha$ MCR and was designated pUNCH319. The larger fragment ligated into pBluescript II SK<sup>+</sup> generated pUNCH320. pUNCH320 caused *E.coli* DH5 $\alpha$ MCR to grow poorly and appeared to be severely restricted in copy number. These data suggested that other sequences located 3' of *frpB* may also be toxic to *E.coli* and that further subcloning was necessary to obtain stable clones. Digestion of pUNCH320 with *Mlu* I and *EcoR* I released fragments of approximately 1.0 kb and 1.5kb, leaving a 2.8kb *Cla* I-*Mlu* I fragment attached to pBluescript II SK<sup>+</sup>. This 5.8kb fragment (vector plus 2.8kb *Cla* I-*Mlu* I insert) was subsequently isolated, treated with Klenow, and re-ligated to itself to generate pUNCH325. DH5 $\alpha$ MCR (pUNCH325) transformants were stable and the plasmid copy number apparently normal.

**Nucleotide sequence and analysis of *frpB*.** PCR amplification of chromosomal DNA followed by sequence analysis of clones confirmed the *Cla* I junction between pUNCH319 and pUNCH325. The combined nucleotide sequence and deduced amino

acid sequence from pUNCH319 and pUNCH325 are shown in Fig. 3. Putative promoter sequences were located upstream of a well conserved Fur box (4). A string of nine cytosine residues was noted between the putative -10 and -35 RNA-polymerase binding sites. A Shine-Dalgarno sequence starting at nucleotide 307 and ending at nucleotide 310 (Fig. 3), was located six bases before an ATG codon, the start of a 1,925bp open reading frame (ORF). This ORF encoded a protein of 713 amino acids. The predicted protein contained a typical signal sequence and characteristic Ala-X-Ala, signal peptidase I cleavage site. The first ten amino acids adjacent to the cleavage site were identical to the peptide sequence obtained from the mature FrpB protein. A classical TonB box was noted at residues 32-36. The mature protein had a calculated molecular weight of 76.6 kD and an isoelectric point of 10.38. The sequence downstream of the ORF revealed an inverted repeat but no string of T residues characteristic of rho-independent transcription termination (69). The protein terminated with an aromatic residue preceded by nine alternating hydrophobic and hydrophilic amino acids. This structure is typical of many bacterial outer membrane proteins sequenced to date (58).

**GenBank homologies.** Comparison of FrpB with other sequences in GenBank revealed some interesting homologies. Several regions of the predicted FrpB protein shared similarity with regions identified in other proteins as potentially important for membrane localization and/or TonB interaction. Localized homology was found between FrpB and the family of TonB-dependent outer membrane receptor proteins including BtuB (25) and FepA (35) of *E.coli* and between Tbp1 (13) and IroA (42) of *Neisseria* species. This similarity was limited to the highly conserved domains (13), and suggested that FrpB may also be a TonB-dependent receptor. More similarity was found with HemR, the hemin receptor of *Yersinia enterocolitica* (55). HemR is an iron-regulated, outer membrane protein that is also a member of the family of TonB-dependent receptor proteins. Overall the two proteins were 26% identical and 48% similar. The most notable similarity was seen with CopB, a major outer membrane

protein of *Moraxella catarrhalis* (26). Overall FrpB and CopB were 52% identical and 71% similar.

**Transposon mutagenesis of *frpB*.** In order to construct FrpB mutants, the

5 gonococcal insert in pUNCH319 was ligated into pUP1(19), creating pUNCH321. The  $\Omega$  fragment from pHP45 $\Omega$  was ligated into a unique *Bgl*I site in pUNCH321 (Insertion site shown in Fig. 3). This DNA was reintroduced into the chromosome of gonococcal strain FA19 by transformation and allelic replacement, creating FA6807. Southern blot analysis of chromosomal DNA from FA19 and FA6807 indicated that a 450bp. MB.3-  
10 hybridizing, *Hinc*II fragment present in the parent was missing in FA6807 and a new reactive band of approximately 2.5kb was present (Fig. 4, panel A). An identical blot (Fig 4, panel B) probed with  $\Omega$ . only hybridized to the 2.5kb fragment in FA6807. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis with anti-FrpB monoclonal antibody W.6, confirmed that FrpB was absent from this strain (Fig.  
15 5).

The  $\Omega$  insertion in *frpB* was also introduced into FA6747 (*tbpA*::mTn3(Cm)) by transformation and allelic replacement creating FA6808. The FrpB<sup>-</sup>/Tbp1<sup>-</sup> phenotype of FA6808 was confirmed by SDS-PAGE and Western blot analysis. This strain was  
20 used for FrpB function analysis as described below.

**Utilization of iron sources.** In an attempt to determine the function that FrpB plays in iron utilization, FA19 and FA6807 were grown in chemically-defined media (CDM) lacking iron. Aliquots of iron-stressed cultures were plated onto CDM agarose  
25 containing 10 $\mu$ M Desferal and GC base agar containing 50 $\mu$ M Desferal. Sterile 3mm discs containing either citrate, transferrin, lactoferrin, heme, hemoglobin, or hemoglobin bound to haptoglobin were positioned around each plate. One disc without any added iron source was added as a negative control. After overnight incubation, growth of both

strains was evident around all discs except the negative control.

*N. gonorrhoeae* can utilize aerobactin (67) and enterobactin (45) as iron sources. To determine if FrpB functioned as either an aerobactin or enterobactin receptor, FA19, FA6808, FA6747, KDF541, KDF541/pABN6, and BN1071 (Table 1) were iron stressed in CDM as above and plated onto CDM agarose containing 2.5 $\mu$ M 30% iron-saturated transferrin. FA6747 and FA6808 could not use Tf as an iron source because they lacked Tbp1, therefore these strains could grow only in the presence of a functional high-affinity siderophore receptor. Three sterile discs were positioned around each plate. Either 30% saturated lactoferrin (positive control for gonococcal viability) or filter-sterilized, iron-free supernatant from LG1315 pColV (aerobactin producer) or AN102 (enterobactin hyper-producer) were added to each disk. After overnight incubation, *E.coli* controls grew as expected suggesting that both siderophores were efficient at stripping iron from transferrin, the sole iron source provided in the media. FA19 grew over the entire transferrin plate as expected, however, growth of FA6808 and FA6747 was only evident around the lactoferrin disks, suggesting that the cells were viable but unable to use aerobactin or enterobactin under these conditions.

Aerobactin utilization by FA19 and FA6807 was further evaluated in chemically-defined, liquid media, employing various concentrations of purified ferri-aerobactin (Fig. 6). The aerobactin receptor-negative *E.coli* strain KDF541 and aerobactin receptor-positive *E.coli* strain KDF541(pABN6) were used as controls. These data suggested that *N. gonorrhoeae* FA19 and FA6807 used ferri-aerobactin similarly and in a concentration-dependent fashion analogous to the aerobactin receptor-negative *E.coli* control. Growth stimulation of gonococci by ferri-aerobactin required relatively high concentrations (3 $\mu$ M) and never attained a density equivalent to that of the Tf or citrate controls. These experiments confirmed the ability of gonococci to utilize ferri-aerobactin as an iron source *in vitro* but showed that this ability was not dependent upon a high-affinity receptor-mediated event.

**<sup>55</sup>Fe uptake from hemin, Tf, and citrate.** Because of the high degree of similarity between HemR, a known hemin receptor in *Y. enterocolitica* and FrpB, it was analyzed whether a quantitative difference in <sup>55</sup>Fe uptake from hemin could be detected between FA19 and FA6807. Uptake of <sup>55</sup>Fe from transferrin by FA19, FA6807, and the Tbp1 mutant FA6747 were used as controls. The results indicated that while <sup>55</sup>Fe uptake from transferrin was approximately wild type in FA6807 (P=.826), <sup>55</sup>Fe uptake from hemin was reduced by approximately 60% (P<0.001) (Fig. 7). Surprisingly, <sup>55</sup>Fe uptake from hemin was also significantly reduced in FA6747 (P<0.001). To determine whether the inability to use <sup>55</sup>Fe from hemin was specific to FA6807 (FrpB<sup>-</sup>) and FA6747 (Tbp1<sup>-</sup>), <sup>55</sup>Fe uptake from hemin was assayed in other well-characterized, gonococcal mutants specifically altered in the expression of other iron-repressible proteins. The Tbp2<sup>-</sup> and Lbp<sup>-</sup> strains, FA6819 and FA6775 respectively, were also reduced in <sup>55</sup>Fe internalization from hemin (P<0.001). These data suggested that either more than one protein was involved in the internalization of hemin iron or the notable decrease in hemin-iron uptake in these mutants resulted from unanticipated, non-specific effects of each of these mutations on a separate membrane-bound, heme-iron-uptake system.

**Reconstruction of *frpB* in pACYC184 and functional complementation of RK1065(*hemA*).** In an attempt to determine if FrpB could function as a heme receptor, an *E. coli hemA* mutant was complemented with FrpB. Although expression of FrpB from the high copy-number vector pBluescript II SK<sup>+</sup> was toxic to *E. coli*, expression from the low copy-number vector pACYC184 was tolerated. The *frpB* reconstruction strategy is outlined in Fig. 8. Briefly, the insert from pUNCH319 was ligated into the *Cla* I and *Bam* H I sites of pACYC184, generating pUNCH330. pUNCH330 was digested with *Cla* I and the gel-purified *Cla* I-*Xba* I fragment from pUNCH325 was ligated into this site as follows. After ligating for four hours, Klenow was added to the ligation mixture for 30 minutes at room temperature to repair non-ligated *Cla* I and *Xba*

I ends. The reaction was further ligated overnight. The *frpB* clone in pACYC184 was designated pUNCH331. FrpB expression from pUNCH331 was iron repressible, suggesting regulation by *E.coli* Fur.

- 5 RK1065 is an *E.coli* *hemA* mutant which is unable to synthesize or internalize heme (27). Growth stimulation requires either  $\delta$ -aminolevulinic acid, or heme and a functional heme receptor. Transformation of pUNCH331 into RK1065 supported growth on heme plates, whereas pACYC184 alone did not (Fig. 9). An Rnase leakage assay was performed to determine if FrpB expression altered the *E.coli* outer membrane, thereby
- 10 allowing heme to simply diffuse into the cell (71). The *E.coli* strains C386 and HB101 containing pEBH21 were used as positive and negative controls respectively. No difference in leakiness was detected between RK1065 (pACYC184) and RK1065 (pUNCH331), suggesting that growth of RK1065 (pUNCH331) on heme plates was not due to a membrane perturbation gross enough to permit leakage of the periplasmic
- 15 protein RNase H. Nevertheless, RK1065 (pUNCH331) was more sensitive to several hydrophobic antibiotics than the same strain with pACYC194 alone (Fig. 9). This experiment suggested that the presence of FrpB in *E.coli* probably allowed heme to enter non-specifically either by creating a pore or by perturbing the integrity of the outer membrane. Uptake of  $^{55}\text{Fe}$  from hemin in RK1065 (pUNCH331) was not inhibited by
- 20 KCN, consistent with a non-specific, non-receptor mediated mechanism of uptake.

- Bactericidal Assay.** In *M. catarrhalis*, CopB, the protein with the greatest similarity to FrpB, appears to play a major role in serum resistance. Mutants which are missing CopB have decreased serum resistance. Mutants which are missing CopB have
- 25 decreased serum resistance and survival in a mouse model (26). Standard bactericidal assays were performed with normal human serum on FA19 and FA6807 grown under iron-limiting conditions and were unable to detect any difference in survival; both strains were completely serum resistant.

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Table 1. Bacterial strains, plasmids and phage.

Strain, plasmid or phage	Description	Source/reference
FA19	Wild type	[Mickelsen, 1981 #38]
FA6807	<i>frpB</i> :: $\Omega$ ( <i>FrpB</i> )	This study
FA6808	<i>frpB</i> :: $\Omega$ <i>thpA</i> ::mTn3(Cm) ( <i>FrpB</i> , <i>Tbp1</i> )	This study
FA6747	<i>thpA</i> ::mTn3(Cm) ( <i>Tbp1</i> )	[Cornelissen, 1992 #13]
FA6819	$\Delta$ <i>thpB</i> ( <i>Tbp2</i> )	[Anderson, 1994 #2]
FA6775	<i>thpA</i> ::mTn3(Cm) ( <i>Lbp</i> )	[Biswas, 1994 #6]
UU1008	Wild type	Zell McGee
DH5 $\alpha$ MCR	<i>F</i> <sup>-</sup> <i>mcrA mcrB mrr</i> $\phi$ 80 <i>dlacZ</i> $\Delta$ M15 $\Delta$ ( <i>argF-lac</i> )U169	Bethesda Research Labs
	<i>recA endA hsdR hsdM supE44</i> $\lambda$ <i>thi-1 gyrA96 relA</i>	
BN1071	<i>F</i> <sup>-</sup> , <i>pro</i> , <i>trp</i> , <i>rslL</i> , <i>entA</i> ( <i>Ent</i> <sup>-</sup> , <i>FepA</i> <sup>+</sup> )	[Klebba, 1982 #30]
AN102	BN1071, <i>leu</i> , <i>sepA</i> ( <i>Ent</i> <sup>+</sup> , <i>FepA</i> <sup>-</sup> )	[Klebba, 1982 #30]
KDF541	BN1071, <i>entA</i> , <i>sepA</i> ( <i>Ent</i> <sup>+</sup> , <i>FepA</i> <sup>-</sup> )	[Rutz, 1992 #46]
KDF541 / pABN6	( <i>Ent</i> <sup>-</sup> , <i>FepA</i> <sup>+</sup> , <i>lutA</i> <sup>+</sup> , <i>luc</i> <sup>-</sup> )	[de Lorenzo, 1987]
LG1315/ pcolV	BN1071, <i>cir</i> ( <i>lutA</i> <sup>+</sup> , <i>luc</i> <sup>+</sup> )	[Warner, 1981 #63]
RK1065	<i>hemA</i>	R. Kadner
HB101	<i>F</i> <sup>-</sup> , <i>hsd20</i> ( <i>r<sub>H</sub></i> , <i>m<sub>H</sub></i> ), <i>recA13</i> , <i>ara-14</i> , <i>proA2</i> , <i>lacY1</i> , <i>galK2</i> , <i>rpsL20</i> ( <i>Sm</i> <sup>r</sup> ), <i>xyl-5</i> , <i>mtl-1</i> , <i>supE44</i> , $\lambda$	Maniatis et. al. 1982
C386	<i>ompA lpp</i>	[Sonntag, 1978 #53]
pACYC184	<i>ori</i> p15a, <i>Cm</i> <sup>R</sup> , <i>Tc</i> <sup>R</sup>	New England Biolabs
pBluescript II SK+	<i>ori</i> pMB1, <i>Ap</i> <sup>R</sup>	Stragene
pHP452	source for the $\Omega$ fragment ( <i>Sm</i> <sup>R</sup> )	[Prentki, 1984 #44]
pUP1	pHSS6 containing gonococcal uptake sequence ( <i>Kan</i> <sup>R</sup> )	[Elkins, 1991 #19]



			WO 96/31618
			[Hardham, 1994 #22]
pEBH21	pBC II SK <sup>+</sup> derivative (Cm <sup>R</sup> )		

pUNCH319	pBluescript II SK <sup>+</sup> containing 540bp <i>EcoR</i> I- <i>Cla</i> I fragment from λfrpB.4	This Study
pUNCH320	pBluescript II SK <sup>+</sup> containing 5.3kb <i>Cla</i> I- <i>EcoR</i> I fragment from λfrpB.4	This Study
pUNCH321	pUP1 containing 540bp <i>EcoR</i> I- <i>Cla</i> fragment from pUNCH319	This Study
pUNCH324	pUNCH321 containing Ω fragment from pHP45Ω in unique <i>Rgl</i> I site	This Study
pUNCH325	pBluescript II SK <sup>+</sup> containing 2.8kb <i>Cla</i> I- <i>Mlu</i> I fragment from pUNCH320	This Study
pUNCH330	540bp <i>EcoR</i> I- <i>Cla</i> fragment from pUNCH319 in pACYC184	This Study
pUNCH331 λ ZapII	reassembled gonococcal <i>frpB</i> gene in pACYC184 excisable lambda phage vector	This Study Statagene

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CLAIMS

## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule that encodes an amino acid sequence comprising a FrpB protein.
2. An isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of Figure 3.
3. An isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of Figure 10.
4. An isolated nucleic acid molecule of claim 1, wherein the FrpB protein is the FrpB protein of *Neisseria gonorrhoeae*.
5. An isolated nucleic acid molecule of claim 1, wherein the FrpB protein is the FrpB protein of *Neisseria meningitidis*.
6. A polypeptide encoded by the isolated nucleic acid molecule of claim 2.
7. A polypeptide encoded by the isolated nucleic acid molecule of claim 3.
8. A vector which comprises the nucleic acid molecule of claim 1.
9. A vector of claim 8, wherein the nucleic acid molecule is linked to a plasmid.
10. A host vector system for the production of a polypeptide having the biological activity of a FrpB antigenic polypeptide which comprises the vector of claim 8 in a

suitable host.

11. A host vector system of claim 10, wherein the suitable host is a bacterial cell or animal cell.
12. A method of producing a polypeptide having the biological activity of a FrpB antigenic polypeptide which comprises growing the host vector system of claim 10 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
13. A method of producing a vaccine composition that protects a mammal from infection by *N. gonorrhoeae* comprising combining the FrpB protein encoded by the isolated nucleic acid of claim 1 with a pharmaceutically acceptable carrier.
14. The method of claim 13 further comprising combining the FrpB with an effective amount of an adjuvant.
15. The method of claim 13, wherein the amino acid sequence of the polypeptide comprises the FrpB protein of *N. gonorrhoeae*.
16. The method of claim 13, wherein the mammal is a human.
17. A method of producing a vaccine composition that protects a mammal from infection by *N. meningitidis* comprising combining the FrpB protein encoded by the isolated nucleic acid of claim 1 with a pharmaceutically acceptable carrier.
18. The method of claim 17 further comprising combining the FrpB with an effective amount of an adjuvant.

19. The method of claim 17, wherein the amino acid sequence of the polypeptide comprises at least a portion of the FrpB protein of *N. meningitidis*.
20. The method of claim 17, wherein the mammal is a human.
21. A vaccine composition capable of protecting a mammal against infection by *N. gonorrhoeae*, the vaccine composition comprising the FrpB protein encoded by the isolated nucleic acid of claim 1 and a pharmaceutically acceptable carrier.
22. The vaccine composition of claim 21 further comprising an effective amount of an adjuvant.
23. The vaccine composition of claim 21, wherein the amino acid sequence of the polypeptide comprises at least a portion of the FrpB protein of *N. gonorrhoeae*.
24. The vaccine composition of claim 21, wherein the mammal is a human.
25. A vaccine composition capable of protecting a mammal against infection by *N. meningitidis*, the vaccine composition comprising the FrpB protein encoded by the isolated nucleic acid of claim 1 and a pharmaceutically acceptable carrier.
26. The vaccine composition of claim 25 further comprising an effective amount of an adjuvant.
27. The vaccine composition of claim 25, wherein the amino acid sequence of the polypeptide comprises at least a portion of the FrpB protein of *N. meningitidis*.
28. The vaccine composition of claim 25, wherein the mammal is a human.

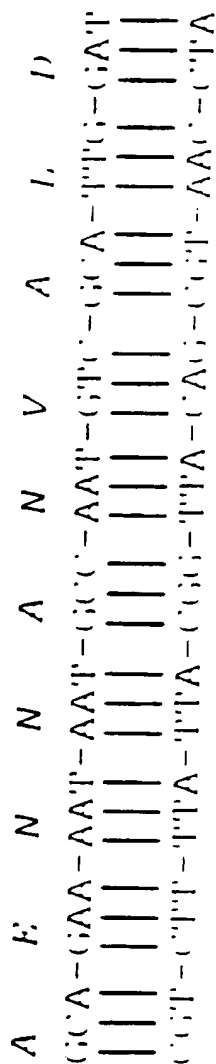


29. A method of protecting a mammal against infection by *N. gonorrhoeae* comprising administering to the mammal a vaccine composition of claim 21.
30. A method of protecting a mammal against infection by *N. meningitidis* comprising administering to the mammal a vaccine composition of claim 25.
31. An antibody directed to an epitope of the FrpB protein encoded by the isolated nucleic acid sequence of claim 2.
32. An antibody directed to an epitope of the FrpB protein encoded by the isolated nucleic acid sequence of claim 3.
33. A method of detecting an antibody specific for *N. gonorrhoeae* in a sample comprising:
- (a) contacting the sample with a FrpB protein encoded by the isolated nucleic acid sequence of claim 4 under conditions to form a complex between the polypeptide and the antibody; and
  - (b) detecting any complex so formed;
- thereby detecting an antibody specific for *N. gonorrhoeae*.
34. A method of claim 33, wherein the FrpB protein is labeled with a detectable marker.
35. A method of detecting an antibody specific for *N. meningitidis* in a sample comprising:
- (a) contacting the sample with a FrpB protein encoded by the isolated nucleic acid sequence of claim 5 under conditions to form a complex between the polypeptide and the antibody; and
  - (b) detecting any complex so formed;

thereby detecting any antibody specific for *N. meningitidis*.

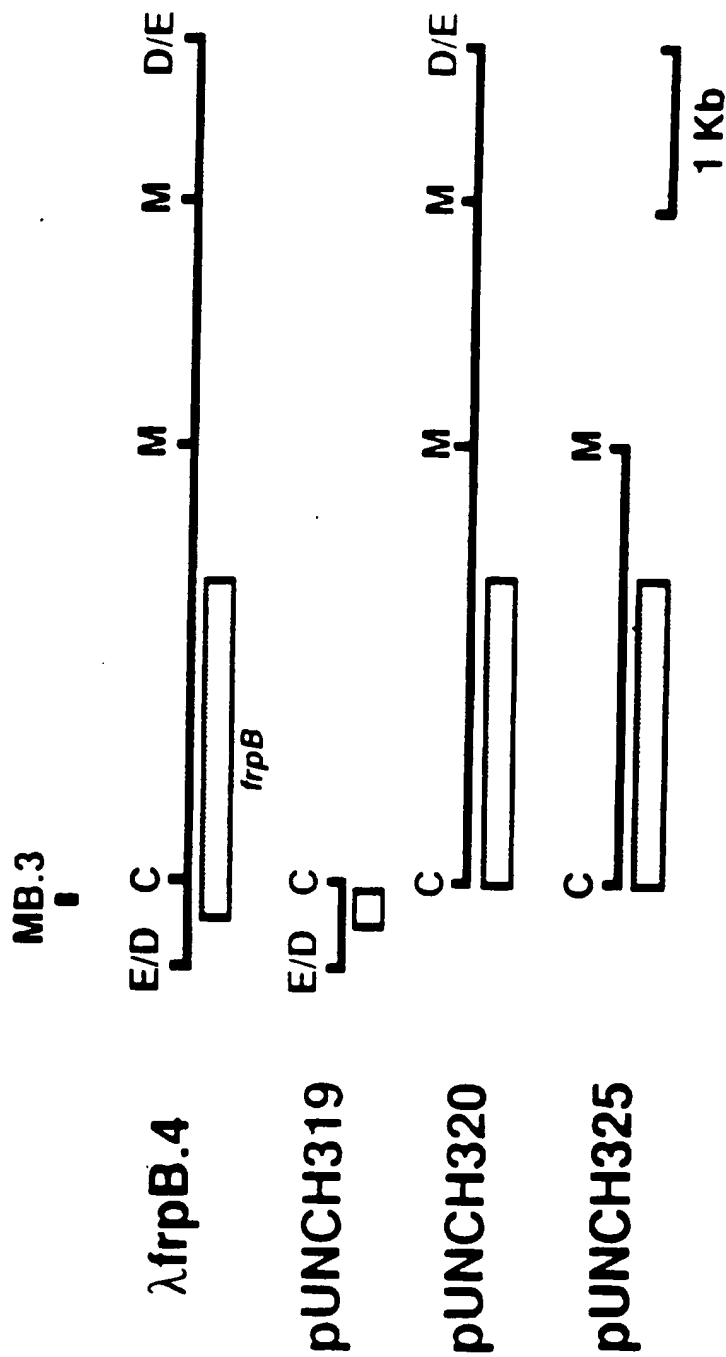
36. A method of claim 35, wherein the FrpB protein is labeled with a detectable marker.
37. A method of treating a mammal infected by *N. gonorrhoeae* comprising administering to the mammal an antibody of claim 31.
38. A method of treating a mammal infected by *N. gonorrhoeae* comprising administering to the mammal an antibody of claim 32.
39. The method of claim 37 or 38 wherein the mammal is a human.
40. The method of claim 37 or 38 wherein the antibody is monoclonal.
41. A method of treating a mammal infected by *N. meningitidis* comprising administering to the mammal an antibody of claim 31.
42. A method of treating a mammal infected by *N. meningitidis* comprising administering to the mammal an antibody of claim 32.
43. The method of claim 41 or 42 wherein the mammal is a human.
44. The method of claim 41 or 42 wherein the antibody is monoclonal.

FIGURE 1

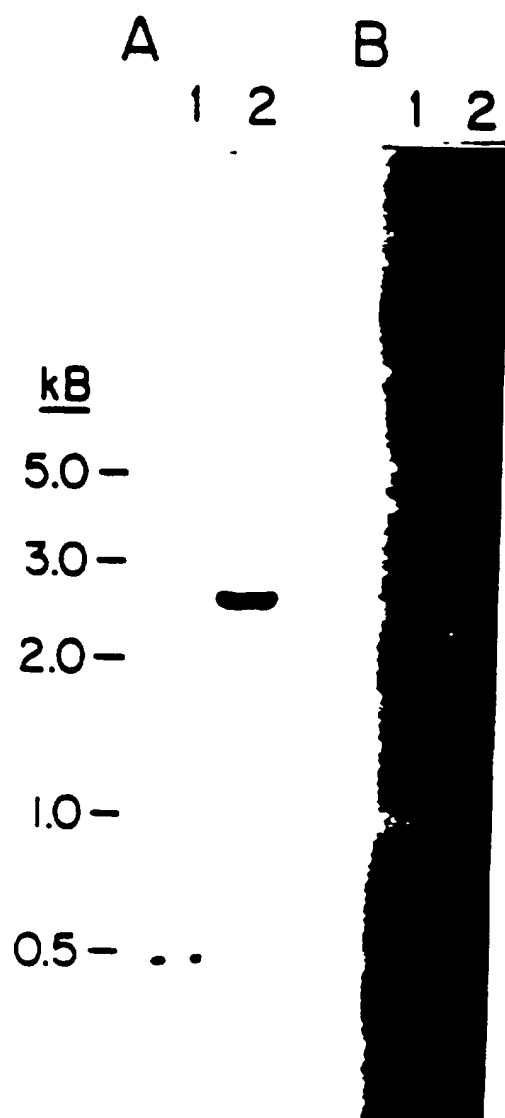


3' **CGI-CTT-TTG-TTG-CGI-TTG-CAI-CG** 5' **MB.3**  
**A A A**

FIGURE 2



101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000



1 2 3 4

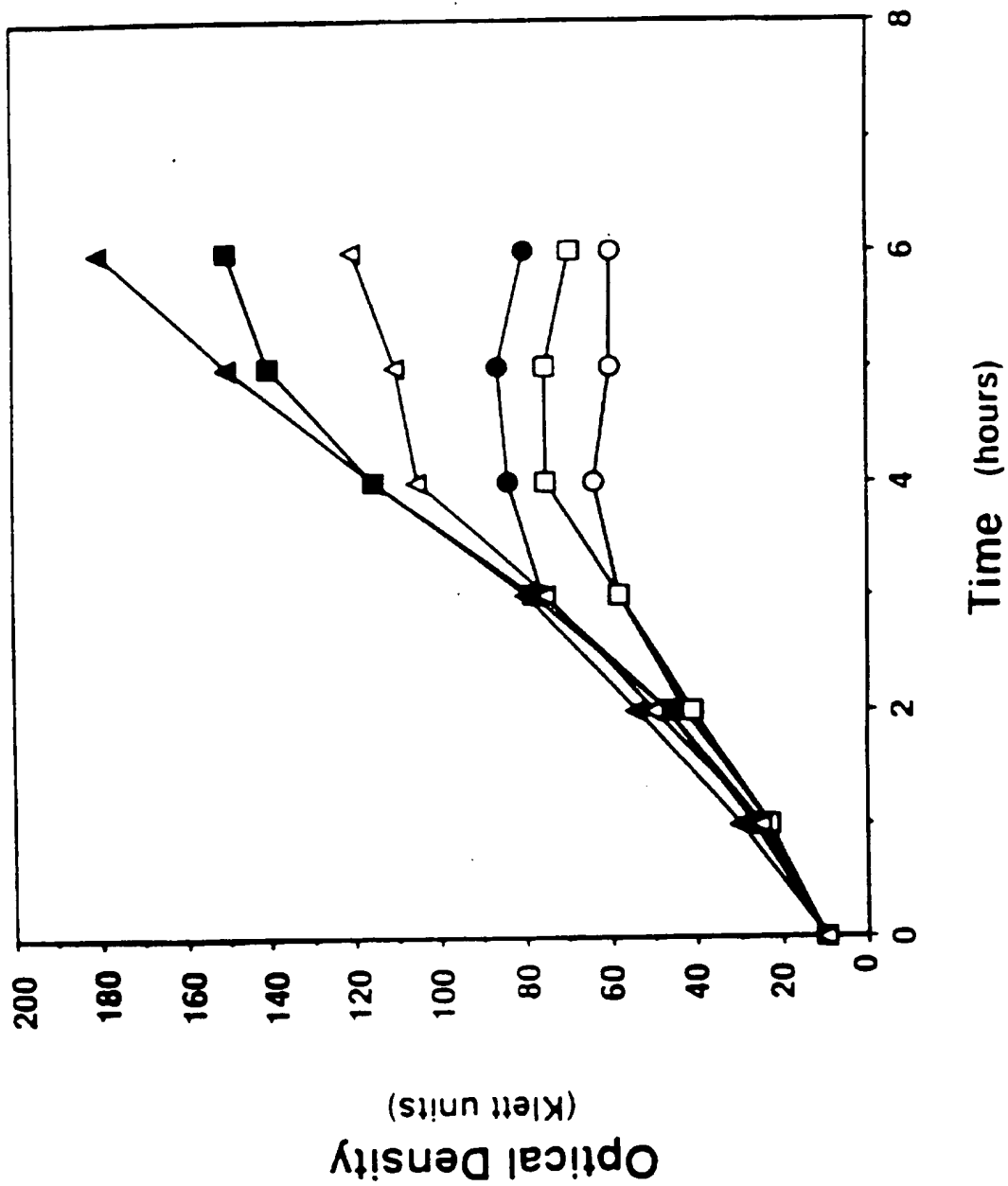
MW

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Figure 6A

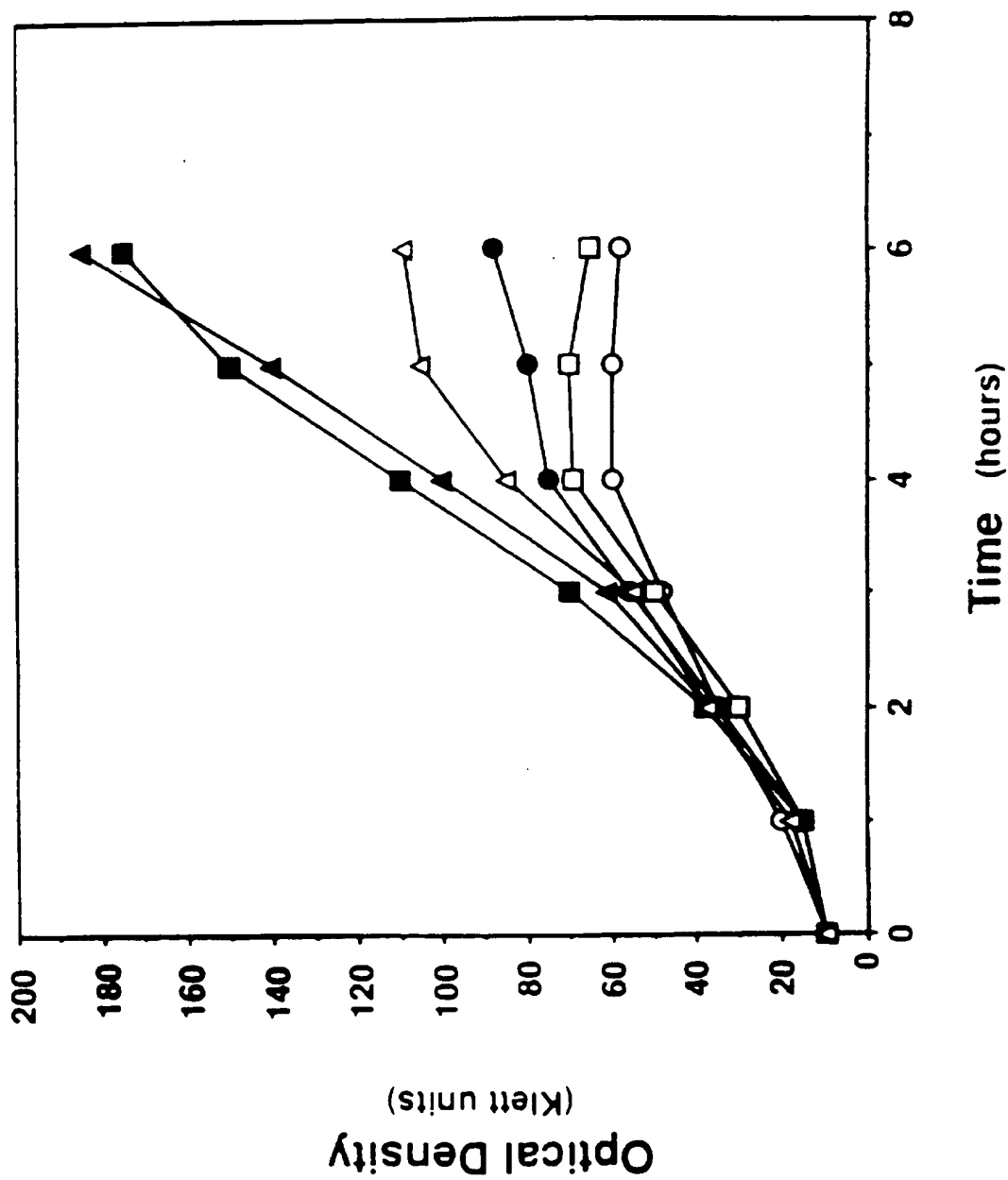


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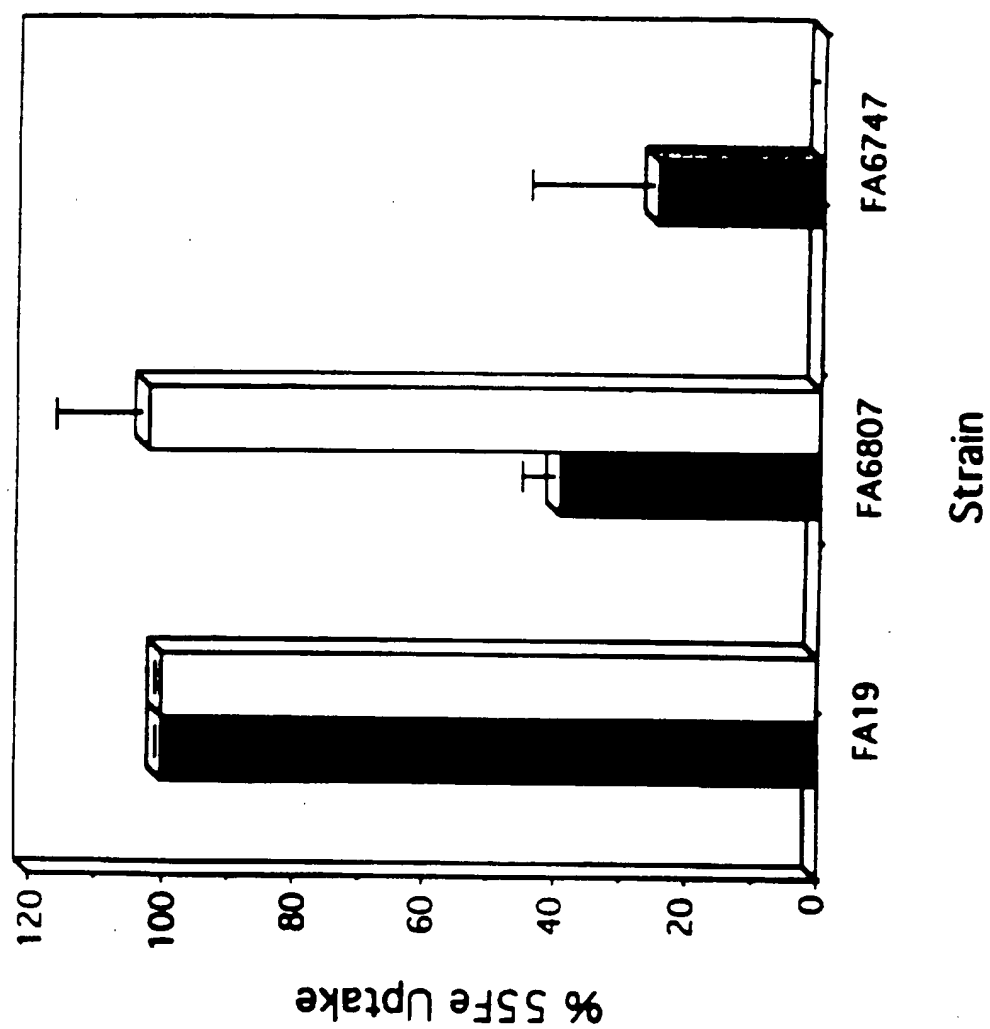
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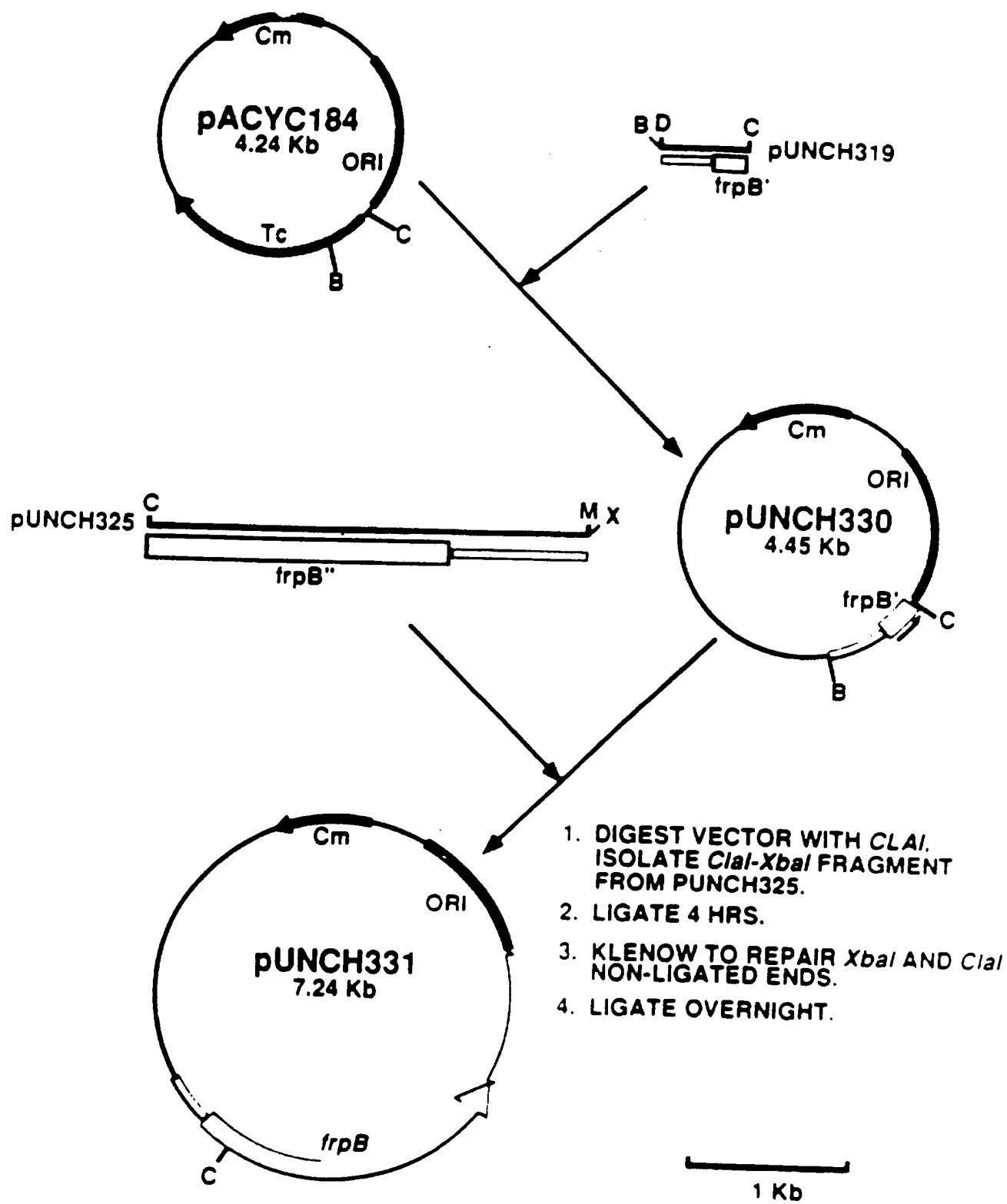
FIGURE 6B

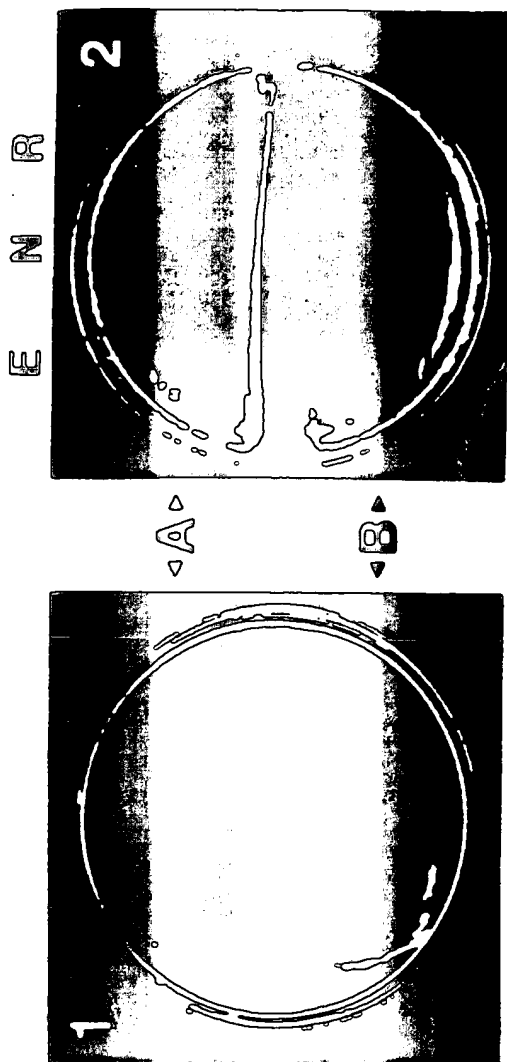


B

FIGURE 7







Sequence Range : to 2369

10 20 30 40 50 60  
AACAAAAAAC AACGCTTCGC AAATAAAAAAC GATAATCAGC TTACACAAAC CCCCCCCCCC  
70 80 90 100 110 120  
GCTAATATAA ACAAATAA TTATTATTAT TTTTCTTAT CCGCCAAAC CTTAACGGT  
130 140 150 160 170  
TGGCTTAAC TCCCTTCATA CACTCAAAAG GACGAACAA ATG AAC GCC GCG TTT TTC  
Met Asn Ala Pro Phe Phe  
TRANSLATION OF ?

180 190 200 210 220  
CGC CTC AGC CTG CTC TCG CTC ACA GTT GCC GCG GGC TTT GCC CAC GCG  
Arg Leu Ser Leu Leu Ser Leu Thr Leu Ala Ala Gly Phe Ala His Ala  
TRANSLATION OF FA1090 CONS DS (A) >

230 240 250 260 270  
GCA GAA AAT AAT GCC AAT GTC GCA TTG GAT ACC GTT ACC GTA AAA GGC  
Ala Glu Asn Asn Ala Asn Val Ala Leu Asp Thr Val Thr Val Lys Gly  
TRANSLATION OF FA1090 CONS DS (A) >

280 290 300 310 320  
GAC CGC CAA GGC AGC AAA ATC GGT ACC AAC ATC GTT ACG GTT CAA CAA  
Asp Arg Gln Gly Ser Lys Ile Arg Thr Asn Ile Val Thr Leu Gln Gln  
TRANSLATION OF FA1090 CONS DS (A) >

330 340 350 360  
AAA GAC GAA AGC ACC GCA ACC GAT ATG GCG GAA CTC TTA AAA GAA GAC  
Lys Asp Glu Ser Thr Ala Thr Asp Met Arg Glu Leu Leu Lys Glu Glu  
TRANSLATION OF FA1090 CONS DS (A) >

370 380 390 400 410  
GCC TCC ATC GAT TTC GGC GGC GGC AAC GCG ACC TCC CAA TTC CTC ACC  
Pro Ser Ile Asp Phe Gly Gly Gly Asn Gly Thr Ser Gln Phe Leu Thr  
TRANSLATION OF FA1090 CONS DS (A) >

420 430 440 450 460  
CTG CGC GGC ATG GGT CAG AAC TGT GTC GAC ATC AAG GTC GAC AAC GCC  
Leu Arg Gly Met Gly Gln Asn Ser Val Asp Ile Lys Val Asp Asn Ala  
TRANSLATION OF FA1090 CONS DS (A) >

470 480 490 500 510  
TAT TCC GAC AGC CAA ATC GTT TAC CAC CAA GCG AGA TTT ATT GTC GAT  
Tyr Ser Asp Ser Gln Ile Leu Tyr His Gln Gly Arg Phe Ile Val Asp  
TRANSLATION OF FA1090 CONS DS (A) >

520 530 540 550 560  
GCC GGT TTG GTT AAA GTC GTT TCC GTA CAA AAA GCG GCG GGT TCC GCC  
Pro Ala Leu Val Lys Val Val Ser Val Gln Lys Gly Ala Gly Ser Ala

\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_>

570 580 590 600  
TCT GCC GGT ATC GGC GCG ACC AAC GCG GCG ATT ATC GCC AAA ACC GTC  
Ser Ala Gly Ile Gly Ala Thr Asn Gly Ala Ile Ile Ala Lys Thr Val>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

610 620 630 640 650  
GAT GCC CAA GAC CTG CTC AAA GCG TTG GAT AAA AAC TGG GCG GTC GCG  
Asp Ala Gln Asp Leu Leu Lys Gly Leu Asp Lys Asn Trp Gly Val Arg>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

660 670 680 690 700  
CTC AAC AGC GCG TTT GCC GCG AAC AAC GCG GTA AGC TAC GCG GCA ACC  
Leu Asn Ser Gly Phe Ala Gly Asn Asn Gly Val Ser Tyr Gly Ala Ser>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

710 720 730 740 750  
GTA TTC GGA AAA GAG GCG AAC TTC GAC GGT TTG TTC TCT TAC AAC GCG  
Val Phe Gly Lys Glu Gly Asn Phe Asp Gly Leu Phe Ser Tyr Asn Arg>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

760 770 780 790 800  
AAC GAT GAA AAA GAT TAC GAA GCG GCG AAA GCG TTC CGC AAT GTC AAC  
Asn Asp Glu Lys Asp Tyr Glu Ala Gly Lys Gly Phe Arg Asn Val Asn>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

810 820 830 840  
GGC GCG AAA ACC GTA CCG TAC AGC GCG CTG GAC AAA CCG AGC TAC CTC  
Gly Gly Lys Thr Val Pro Tyr Ser Ala Leu Asp Lys Arg Ser Tyr Leu>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

850 860 870 880 890  
GCC AAA ATC GGA ACA ACC TTC GCG GAC GCG GAC CAC CGC ATC GTA TTG  
Ala Lys Ile Gly Thr Thr Phe Gly Asp Gly Asp His Arg Ile Val Leu>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

900 910 920 930 940  
AGC CAT ATG AAA GAC CAA CAC CGG GCG ATC GCG ACT GTG GGT GAA GAG  
Ser His Met Lys Asp Gln His Arg Gly Ile Arg Thr Val Arg Glu Glu>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

950 960 970 980 990  
TTT GCG GTC GCG GCG GAA AAT TCA CCG ATA ACT ATT AAA CCG CAA GCC  
Phe Ala Val Gly Gly Glu Asn Ser Arg Ile Thr Ile Lys Arg Gln Ala>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

1000 1010 1020 1030 1040  
CCT GCC TAC CCG GAA ACC ACA CAA TCG AAC ACC AAT TTG GCG TAC ACC  
Pro Ala Tyr Arg Glu Thr Thr Gln Ser Asn Thr Asn Leu Ala Tyr Thr>  
\_\_\_a\_\_\_a\_\_\_a\_\_\_ TRANSLATION OF FA1090 CONS DS (A)\_\_\_a\_\_\_a\_\_\_a\_\_\_>

1530 1540 1550 1560

AGT TGC GTG ATT TGC CAG GCG TGC GAA CAG TGC TGC ACC GCG  
Ser Phe G. Val Ile Trp Glu Pro Arg Glu His Trp or Phe Ser Ala>  
\_a\_a\_a\_TRANSLATION OF FA1090 CONS DS (A)\_a\_a\_a\_>

1570                1580                1590                1600                1610

AGC CAC AAC TAC GCC AGG CGG AGC GCG CGC CTG TAT GAC GCG CTG CAA  
Ser His Asn Tyr Ala Ser Arg Ser Pro Arg Leu Tyr Asp Ala Leu Glu>

\_\_\_\_\_a\_\_\_\_a\_\_\_\_a\_\_\_\_\_TRANSLATION OF FALL90 CONS DS [A]\_\_\_\_\_a\_\_\_\_a\_\_\_\_a\_\_\_\_\_

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1520      1630      1640      1650      1660
ACC CAC GGC AAA CGC GGC ATC ATC TCG ATT GGC GAC GGC ACC AAA GGC
Thr His Gly Lys Arg Gly Ile Ile Ser Ile Ala Asp Gly Thr Lys Ala>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS [A]_a_a_a_a_

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1670 1680 1690 1700 1710  
GAA CGC GCG CGC AAT ACC GAA ATC GGC TTC AAC TAC AAC GAC GGC ACC  
Glu Arg Ala Arg Asn Thr Glu Ile Gly Phe Asn Tyr Asn Asp Gly Thr>  
-----TRANSLATION OF PA199 CONS DS (A)-----

1720            1730            1740            1750            1760

TTT GCC GCA AAC GGC AGC TAC TTC CGG CAG ACC ATC AAA GAC GCG CTG  
Phe Ala Ala Asn Gly Ser Tyr Phe Arg Glu Thr Ile Lys Asp Ala Leu>

\_\_\_\_\_a\_\_\_\_a\_\_\_\_\_TRANSLATION OF FA1890 CONS DS [A]\_\_\_\_\_a\_\_\_\_\_a\_\_\_\_\_>

1770 1780 1790 1800

GCC AAT CCG GAA AAC CGC CAC GAC TGT GTC GCC GTC CGC GAA GCC GTC  
Ala Asn Pro Gln Asn Arg His Asp Ser Val Ala Val Arg Gln Ala Val>

-----TRANSLATION OF FA190 CONS DS [A]-----

1810                    1820                    1830                    1840                    1850

AAC GCC GGC TAC ATC AAA AAC CAC GGT TAC GAA TTG GGC GCG TCC TAC  
Asn Ala Gly Tyr Ile Lys Asn His Gly Tyr Glu Leu Gly Ala Ser Tyr>

-----TRANSLATION OF FA190 CONS DS (A)-----

1860                      1870                      1880                      1890                      1900

CGC ACC GGC GGC CTG ACC GCC AAA CTC    GGC GTA AGC    CGC AGC AAA CCG  
Arg Thr Gly Gly Leu Thr Ala Lys Val    Gly Val Ser Arg Ser Lys Pro>

-----TRANSLATION OF FALL 1961-----CONS DS [A]-----

1910                      1920                      1930                      1940                      1950

CGC TTT TAC GAT ACC CAT CCT AAA AAA CTG TTG AGC GCG AAC CCC GAG  
Arg Phe Tyr Asp Thr His Pro Lys Lys Leu Leu Ser Ala Asn Pro Glu>

\_\_\_\_\_TRANSLATION OF PAL90 CONS ES (A)\_\_\_\_\_>

1960	1970	1980	1990	2000
TTT GGC GCA CAA ACC GGC CGC ACT TGG	ACC GCC	TCC CTT GCC TAC GCG		
Phe Gly Ala Gin Thr Gly Arg Thr Trp	Thr Ala	Ser Leu Ala Tyr Arg>		
<u>a_a_a_a_a</u> TRANSLATION OF FAL990 CONS DS [A] <u>a_a_a_a_a</u>				

2010			2020			2030			2040						
TTC	AAA	AAC	CGG	AAT	CTG	GAA	ATC	GCC	TGG	CGC	GGA	CGC	TAT	GTT	CAA
Phe	Lys	Asn	Pro	Asn	Leu	Glu	Ile	Gly	Trp	Arg	Gly	Arg	Tyr	Val	Gln



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_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2050      2060      2070      2080      2090
AAA GCT ACC GGT TCG ATA TCG GCG GCA GCG CAA AAA GAC GCG GAC GCG
Lys Ala Thr Gly Ser Ile Leu Ala Ala Gly Gln Lys Asp Arg Asp Gly>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2100      2110      2120      2130      2140
AAA TTG GAA AAC GTT GTA CGC CAA GGT TTC GGT GTC AAC GAT GTC TTC
Lys Leu Glu Asn Val Val Arg Gln Gly Phe Gly Val Asn Asp Val Phe>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2150      2160      2170      2180      2190
GCC AAC TGG AAA CCG CTG GGC AAA GAC ACG CTC AAT GTT AAT CTT TCG
Ala Asn Trp Lys Pro Leu Gly Lys Asp Thr Leu Asn Val Asn Leu Ser>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2200      2210      2220      2230      2240
GTT AAC AAC GTG TTC GAC AAG TTC TAC TAT CCG CAC AGC CAA CGC TGG
Val Asn Asn Val Phe Asp Lys Phe Tyr Tyr Pro His Ser Gln Arg Trp>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2250      2260      2270      2280
ACC AAT ACC CTG CCG GGC GTG GGA GGT GAT GTA CGC CTG CGC GTG AAC
Thr Asn Thr Leu Pro Gly Val Gly Arg Asp Val Arg Leu Gly Val Asn>
_a_a_a_a_TRANSLATION OF FA1090 CONS DS (A)_a_a_a_a_>
2290      2300      2310      2320      2330      2340
TAC AAG TTC TAA AACGCACAT CCGGAAAAAA TCGCGTGTGA AAGCGTTTCA
Tyr Lys Phe ***>
__TRANSLAT__>
2350      2360
GACGGCATCT GTCCGTGATAA TTGGATATA

```

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/04774

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.6, 7.32; 530/380, 388.25, 388.4, 389.3, 389.5; 536/23.7; 424/249.1, 250.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE

search terms: FrpB protein, vaccine, N. gonorrhoeae, N. meningitidis

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Vaccine, Vol 12 No 6, issued 1994, Ala'Aldeen et al, "Vaccine Potential of meningococcal FrpB: studies on surface exposure and functional attributes of common epitopes", pages 535-541, see pages 535 and 538.	1-36 --- 37-44
X, P --- Y, P	Dissertation Abstract International, Vol 56 No 2, issued August 1995, Beucher, M., "Cloning, sequencing and characterization of the gene encoding FrpB, a major iron-regulated outer membrane protein of Neisseria gonorrhoeae", page 624, see entire document.	1-2, 4, 6, 8-12 --- 3, 5, 7, 13-44



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 JULY 1996

Date of mailing of the international search report

25.07.96

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/04774

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Infection and Immunity, Vol 56 No 4, issued April 1988, Dyer et al, "A pleiotropic iron-uptake mutant of Neisseria meningitidis lacks a 70-kilodalton iron-regulating protein", pages 977-983, see page 980.	6-7, 31-32 --- 1-5, 8-30, 33-44
X, P --- Y, P	Infection and Immunity, Vol 63, No 10, issued October 1995, Pettersson et al, "Molecular Characterization of FrpB, the 70-kilodalton iron-regulated outer membrane protein of Neisseria meningitidis", pages 4181-4184, see page 4182.	1, 3, 5, 7-12 --- 2, 4, 6, 13-44

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/04774

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12P 21/04, 21/08; A61K 35/18, 38/00; C07K 1/00, 14/195, 16/12; C07H 21/04; A61K 39/095

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.6, 7.32; 530/380, 388.25, 388.4, 389.3, 389.5; 536/23.7; 424/249.1, 250.1

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